Carotenoids
Volume 5: Nutrition and Health

Chapter 3

Carotenoids in Food

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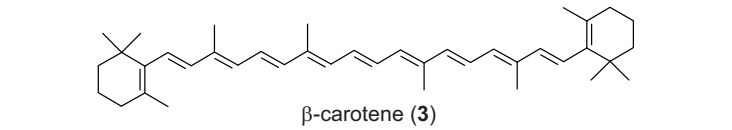
A. Introduction

No members of the animal kingdom, including humans, can synthesize carotenoids. Even those animals (birds, fish, invertebrates) that use carotenoids for colouration must obtain them from the diet. Although humans, being mammals, are normally not coloured by carotenoids, analysis of human blood and tissues reveals a significant content of carotenoids which, as discussed later in this book, are associated with good health and reduced risk of diseases. Although some carotenoids are added to manufactured foods as colourants, or are taken as supplements (*Chapter 4*), most ingested carotenoid is obtained direct from natural food, especially vegetables and fruit.

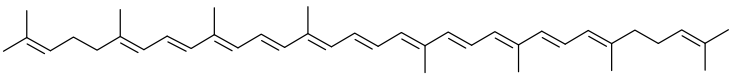
In richer countries, where food is plentiful, much publicity is given to the possible benefits of a carotenoid-rich diet to maintain health and reduce risks of serious age-related degenerative diseases and conditions such as cancer, coronary heart disease and macular degeneration, as discussed in later *Chapters* in this *Volume*. Attention is focused on encouraging the consumption of 'healthy foods' or 'functional foods' which provide high intake of the carotenoids of interest. The target is to have dietary sources that provide a high concentration of those carotenoids, notably β-carotene (**3**), lycopene (**31**), lutein (**133**), zeaxanthin (**119**) and β-cryptoxanthin (**55**), which have been investigated most for an association with beneficial effects.

A large proportion of the world's population live in poverty and don't have the luxury of living long enough to develop these diseases. For people who live in poorer countries, the priority need for carotenoids is different but acute. The essential nutrient vitamin A is a metabolite of the provitamins β-carotene and some related carotenoids, notably α-carotene (**7**)

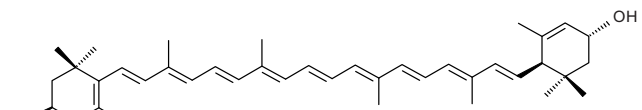
and β-cryptoxanthin (**55**), and these carotenoids provide most of the vitamin A for many populations in the world. In countries where vitamin A deficiency is a real or potential problem, the availability and provision of food containing sufficient amounts of provitamin A carotenoids, especially β-carotene, can be a matter of life or death (see *Chapter 9*).



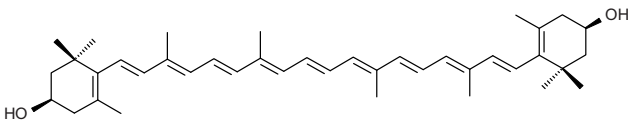
β-carotene (**3**)



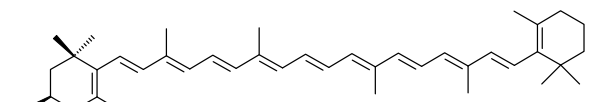
lycopene (**31**)



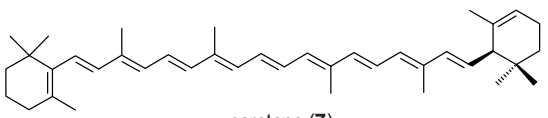
lutein (**133**)



zeaxanthin (**119**)



β-cryptoxanthin (**55**)



α-carotene (**7**)

In the context of both rich and poorer countries, knowledge of carotenoid content and composition is therefore essential in order that guidance can be given on what food sources can provide adequate supplies of desired carotenoids.

Over many years, thousands of papers have been published describing carotenoid content and composition of particular species and varieties under different conditions. The literature is

flooded with numbers, reporting precise carotenoid concentrations, obtained by different analytical methods, especially HPLC (*Chapter 2*). The great variation in results can be extremely confusing. A major aim of this *Chapter* is to plot a way through this confusion and give some realistic evaluation and guidance.

The most important sources of carotenoids in the human diet are vegetables and fruit. The overall contribution of animal-derived food products is not large, but it must not be overlooked. Dairy products, eggs and some fish and seafood can have a significant carotenoid content. Also synthetic and natural carotenoids and carotenoid-rich extracts are widely used as natural colourants in manufactured food products such as cakes, confectionery, ice-creams and drinks.

Cultivation practices and methods of cooking and processing food vary widely around the world, and can have a profound effect on the stability and therefore the content of carotenoids.

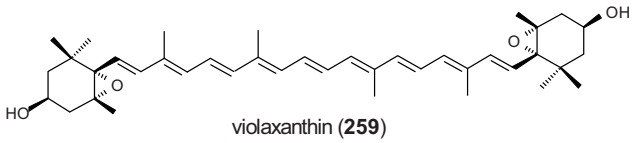
B. Distribution of Carotenoids in Vegetables and Fruits

There can be some confusion over the description of a food as a fruit or a vegetable. Anatomical accuracy and culinary usage often do not coincide. Tomato and pepper, for example, are clearly fruits but are generally used as vegetables. In some reviews, the term 'fruit vegetables' has been used for such examples [1]. Also some foods eaten as vegetables are actually flowers (broccoli, cauliflower) or seeds and seed-bearing structures (peas, beans).

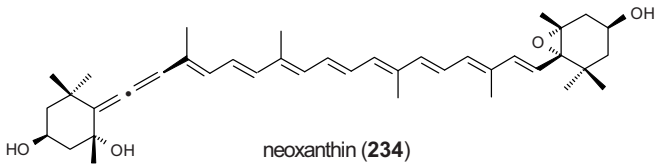
The ability to synthesize and accumulate carotenoids is determined genetically, but actual carotenoid compositions and contents are also highly dependent on environmental and cultivation conditions (Section **C**).

1. Green vegetables and fruits

All green plant tissues, not only leaves and stems but also green fruit and pods and seeds of legumes such as beans and peas, are green because of the presence of chlorophyll in the photosynthetic structures, the chloroplasts (see *Volume 4, Chapter 14*). In chloroplasts the chlorophyll-containing pigment-protein complexes of photosystems 1 and 2 also contain carotenoids. The carotenoid composition of plant chloroplasts is remarkably constant with, as the main components, β-carotene (25-30% of the total), lutein (40-50%), violaxanthin (**259**) (15%) and neoxanthin (**234**) (15%).

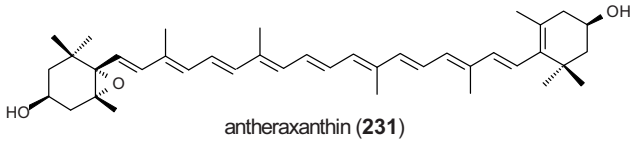


violaxanthin (**259**)

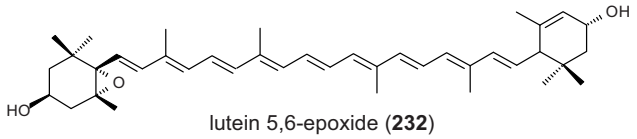


neoxanthin (**234**)

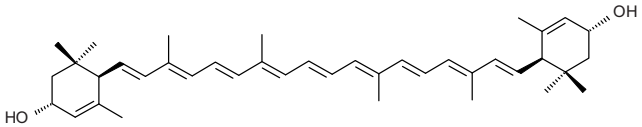
Small amounts of other carotenoids may be detectable, namely α-carotene, zeaxanthin, antheraxanthin (**231**) and lutein 5,6-epoxide (**232**). Very rarely, the only frequently consumed example being lettuce, some of the lutein may be replaced by lactucaxanthin (**150**).



antheraxanthin (**231**)



lutein 5,6-epoxide (**232**)



lactucaxanthin (**150**)

Although the carotenoid composition is almost constant, the quantitative carotenoid contents vary widely. There is, though, a clear correlation; darker green indicates a high population of chloroplasts and therefore a high concentration of carotenoids. In vegetables such as lettuce and members of the cabbage family which consist of more-or-less tightly packed leaves, the darkest green and hence the highest carotenoid concentration is in the outer leaves. Inner leaves that are not exposed to light may be pale green or almost white, with very little carotenoid, or may be yellow (etiolated) and have a different carotenoid composition, usually having little or no β-carotene and an altered xanthophyll composition. This variation must be taken into account when the carotenoid profile and content of a particular vegetable that is being consumed is estimated.

The reversed-phase HPLC profile of an extract from Brussels sprouts was illustrated in *Chapter 2*, *Fig. 3*. Other green fruits and vegetables have similar HPLC profiles [2] and some, *e.g.* green beans and lima beans show the additional presence of considerable amounts of α-carotene [2].

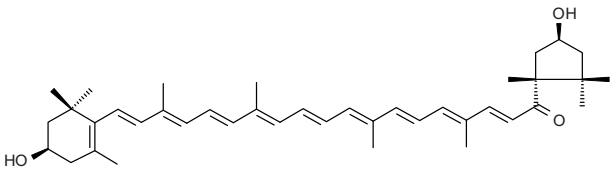
The qualitative distribution of the major carotenoids in some of the most commonly consumed green fruits and vegetables has been published in a review [3]. In all cases, these carotenoids are accompanied by considerable amounts of their geometrical isomers. For symmetrical carotenoids, the most common geometrical isomers in foods are 9*Z* and 13*Z* and, to a lesser extent, the 15*Z* isomer. With the unsymmetrical carotenoids, 9*Z*, 9′*Z*, 13*Z*, 13′*Z*, and 15*Z* isomers may all be present in variable concentrations. The occurrence of di-*Z* isomers of carotenoids in foods is rare.

2. Yellow, orange and red fruits and vegetables

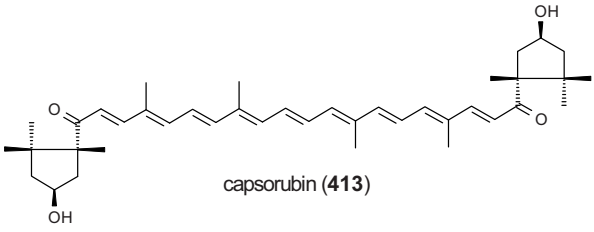
Many of the richest sources of carotenoids are not green. Yellow, orange and red plant tissues, including fruits, flowers, roots and seeds, may contain high concentrations. It is important to realise, however, that these colours are not always due to carotenoids; anthocyanins, betalains and quinones provide other striking examples.

a) Fruits

Fruit represent a major dietary source of carotenoids and have been studied extensively. Although some fruits contain insignificant amounts of carotenoids or small amounts of the carotenoids that are usually found in chloroplasts, others contain larger amounts of different carotenoids. Some distinctive patterns have been recognized [4,5] that appear in a range of fruits: (i) large amounts of the acyclic carotene lycopene, as in tomatoes (red colour), (ii) large amounts of β-carotene and/or its hydroxy derivatives β-cryptoxanthin and zeaxanthin (orange colour), (iii) as (ii) but with also α-carotene and/or its hydroxy derivatives, especially lutein (yellow-orange), (iv) large amounts of carotenoid epoxides (yellow), and (v) carotenoids that appear to be unique to or characteristic of that species (yellow, orange or red), *e.g.* capsanthin (**335**) and capsorubin (**413**) in red peppers (*Capsicum annuum*).

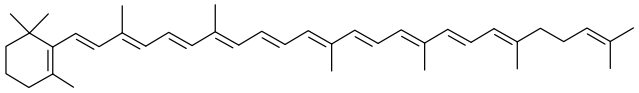


capsanthin (**335**)

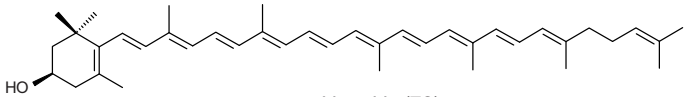


capsorubin (**413**)

Now that the genes of carotenoid biosynthesis are known and understood, these observations can be rationalized (see *Volume 3, Chapters 2* and *3*). Green, unripe fruits contain chloroplasts in which the usual collection of chloroplast carotenoids is found. As the fruits mature, these chloroplast pigments may remain or may be degraded. In many cases, however, familiar colour changes take place as the fruits ripen and develop chromoplasts, sub-cellular organelles that replace chloroplasts and may be derived from them. The carotenoids are biosynthesized and accumulate in the chromoplasts. The biosynthesis is controlled by a set of genes which are activated as a key feature of the ripening process. The carotenoid composition of the ripe fruit is determined by which ripening-specific genes are present and activated. So if the phytoene synthase and desaturase genes are active, lycopene will be produced (category i), if in addition the β-cyclase and hydroxylase genes are active, the dicyclic β-carotene and its hydroxy derivatives or the moncyclic γ-carotene (**12**) and its hydroxy derivative rubixanthin (**72**) will accumulate (category ii). When the ε-cyclase and ε-hydroxylase are also present, α-carotene and lutein are produced (category iii). Similarly, an active epoxidase gene gives category iv. Additional genes may also be present, leading to other end-products (category v).



γ-carotene (**12**)



rubixanthin (**72**)

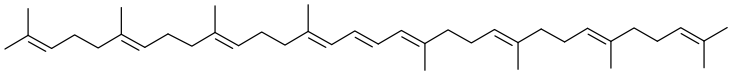
Application of this knowledge makes possible the genetic modification of carotenoid profiles or content in various crop plants (for details see *Chapter 6*).

Some yellow/red fruits and vegetables contain mostly carotenes and only small amounts of xanthophylls [6,7]. With some of these, *e.g.* apricot, the carotenoid profile, illustrated in *Chapter 2*, *Fig. 5*, is complicated, consisting not only of α-carotene and β-carotene but also containing a range of biosynthetic intermediates and *Z* isomers.

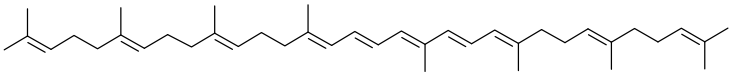
Other examples, *e.g.* the root vegetables carrot and sweet potato, have a simpler HPLC profile with only α-carotene and β-carotene and small amounts of biosynthetic intermediates present. The qualitative distribution of the major carotenoids in commonly consumed yellow fruits and vegetables has been published [3].

Some red fruits, *e.g.* tomato, are major dietary sources of lycopene and the biosynthetic intermediates phytoene (**44**), phytofluene (**42**) and ζ-carotene (**38**), and to a lesser extent also contain neurosporene (**34**), β-carotene, and γ-carotene [7-9]. The lycopene content of

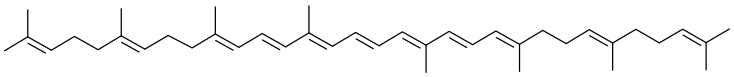
tomatoes can reach 5-10 mg/100g. This high content is maintained in tomato-based food products such as ketchup, soup and sauces [3,8-10].



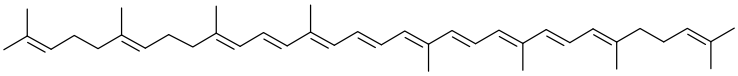
phytoene (**44**)



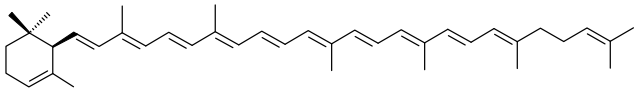
phytofluene (**42**)



ζ-carotene (**38**)



neurosporene (**34**)



δ-carotene (**21**)

Other commonly consumed fruits that contain lycopene are pink grapefruit, water melon, papaya and, in low concentration, apricots (fresh, canned, dried) [7]. A typical carotenoid HPLC profile of an extract from tomato paste is shown in *Chapter 2*, *Fig. 6*.

Many strains and mutants of tomato have been produced with quite different carotenoid profiles, *e.g.* 'high-beta' and 'high-delta' strains in which the lycopene is replaced by high concentrations of β-carotene or δ-carotene (**21**), respectively. The genetic modification of carotenoid content and composition in tomatoes is discussed in *Chapter 6*.

Yellow/orange fruits and vegetables, *e.g.* mango, papaya, peaches, prunes, acorn and winter squash, and oranges [3,7,10-12], may contain, in addition to carotenes and often as the main pigments, xanthophylls and xanthophyll epoxides, esterified with straight chain fatty acids such as lauric, myristic, and palmitic acids [3,7,10-12]. Strains of squash (*Cucurbita maxima*) that contain a high concentration of esters of lutein and other xanthophylls have been studied extensively. There are significant differences in qualitative distribution of carotenoids and their esters in different cultivars. As shown in *Chapter 2, Fig. 8*, the major carotenoids in

the 'butternut' squash are α-carotene, β-carotene, and lutein diacyl esters but not monoesters, whereas the 'Northrup King' squash contains mainly lutein and its monoesters and α-carotene is absent (*Chapter 2, Fig. 7*). In acorn squash, violaxanthin and its monoesters and diesters are also present.

Small amounts of lutein dehydration products are detected in squash but are rarely found elsewhere [13]. Their general occurrence in human plasma is attributed to metabolism of dietary lutein.

Citrus fruits, *e.g.* oranges, tangerines, grapefruit and lemons, and their juices, are widely consumed. They have been studied extensively and many different varieties, strains and hybrids analysed [14]. The carotenoid compositions are very variable and can be complex. β-Carotene, β-cryptoxanthin and violaxanthin are characteristic and apocarotenoids are common, sometimes as the main pigments. The carotenoids are present not only in the brightly coloured peel but also in internal tissues and juice. The carotenoid compositions of the different parts can differ considerably. The carotenoid content and composition of the juices depend on which parts of the fruit have been used in the processing.

b) Roots

In roots such as carrots and sweet potatoes that contain a high amount of carotene, the pigments are also synthesized and accumulate in chromoplasts. In carrots, the concentrations accumulated and the ratio of α-carotene to β-carotene vary considerably between strains [15]. α-Carotene can range from 5% to 50% of the total carotene. Varieties with a deeper orange-red hue have a higher proportion of β-carotene. The concentration in outer tissues (phloem) is generally greater than that in the inner core (xylem). Some red varieties also contain lycopene, which can be the main pigment. A yellow variety has a considerable concentration of lutein instead of β-carotene. Sweet potatoes also accumulate β-carotene, usually with little α-carotene. Common potatoes, even yellow varieties, contain only low levels of the common chloroplast xanthophylls. Genetically modified potatoes, engineered to accumulate carotene, have been produced (see *Chapter 6*). Other yellow root vegetables may contain carotenoids in low concentration, including, in swede (*Brassica rutabaga*) some lycopene [16].

c) Seeds

The yellow-orange colour of the outer coat of sweetcorn (maize, *Zea mays*) is due primarily to lutein, β-carotene, zeaxanthin and cryptoxanthins [17].

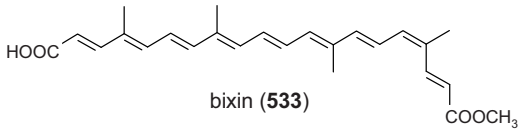
An extensive programme has led to the development of a genetically modified 'Golden' rice [18], that accumulates β-carotene in the butter-coloured endosperm (see *Chapter 6*).

Wheat and pasta products have been analysed [19]. The only carotenoids of note present were lutein and zeaxanthin, with lutein predominating. The concentrations were generally very low (ng/g) though somewhat higher (μg/g) in an Australian green-harvested wheat. The

carotenoid content of wheat pasta is rather higher because of lutein and zeaxanthin from eggs used in the processing. Pasta made from durum wheat (*Triticum durum*) also contains more lutein from the durum flour. There is very little carotenoid in other cereals and flours.

Green seeds of legumes *e.g*. peas, contain β-carotene and chloroplast xanthophylls [4].

The seed coat of the shrub *Bixa orellana* accumulates an extremely high concentration of the apocarotenoid bixin (**533**). This product is widely used as a food colourant (annatto) but it is not known if it is of any consequence for human health.



bixin (**533**)

d) Flowers

Flowers are not widely consumed, the best known examples being cauliflower and broccoli (*Brassica oleracea*, cv. group Botrytis and Italica, respectively). Familiar white cauliflowers contain little or no carotenoid, but an orange strain, accumulating β-carotene, first found in a field of white cauliflowers, is now available for growing commercially [20]. The flower heads of broccoli and calabrese are harvested before the petals open. In older or stored examples the yellow florets, rich in lutein esters, may start to show.

e) Oils

Fruit of some oil palms synthesize and accumulate a high concentration of α-carotene and β-carotene which are retained in the oil from the pressed fruit. Red palm oil from *Eleais guineensis* is processed on a large scale and refined in various forms for use as a cooking oil and ingredient in manufactured foods [21]. Some other palm fruits, *e.g*. the South American 'Buriti' (*Mauritia vinifera*) also have a very high carotenoid content [22].

Canola (rapeseed, *Brassica napus*) oil normally contains little or no carotenoid but the ability to produce carotenoids can be introduced by GM methods [23].

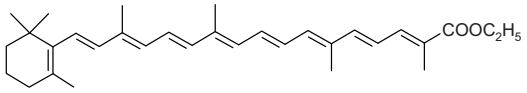
The oil of the South-East Asian 'Gac' fruit (*Momordica cochinchinnensis*) contains a high concentration of β-carotene [24] but has so far found only local use.

3. Animal-derived food products

Animals do not biosynthesize carotenoids but many can accumulate, sometimes in quite high concentration, carotenoids that they ingest. If such animal tissues or products are eaten as part of the human diet, they provide an additional source of carotenoids. There are well known examples of this.

a) Eggs

The yellow colour of egg yolk is due to carotenoids. The colour hue and intensity depend on the poultry feed used (see *Volume 4, Chapter 13*). Marigold flowers or lutein esters produced from them are widely used in chicken feed, so the eggs provide a good source of lutein. The more orange-yellow yolks from corn-fed hens also contain zeaxanthin. Some synthetic apocarotenoids, such as 8′-apo-β-caroten-8′-oic acid (**486**) ethyl ester are also used as additives to give a more orange hue. These apocarotenoids have provitamin A activity.



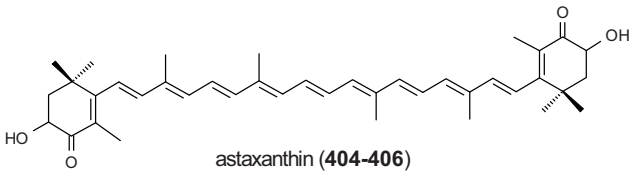
8'-apo-β-caroten-8'-oic acid (**486**) ethyl ester

b) Dairy produce

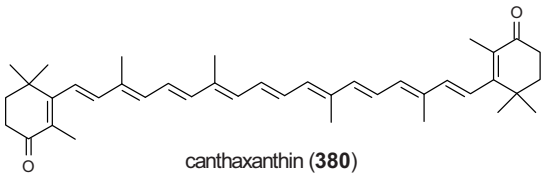
Cattle specifically absorb carotenes and not xanthophylls. This carotene may colour the fat yellow, and is also present in milk fat. Milk, cream, butter and cheese are therefore likely to contain some β-carotene, though the concentration is usually not great [14]. The presence of carotene often varies with season; it is highest in the early summer when the animals are grazing the best quality pastures.

c) Seafood

Pink-fleshed fish, notably salmon and trout, accumulate in the muscle high concentrations of astaxanthin (**404-406**) or canthaxanthin (**380**) that they obtain from their natural food or which is added to their feed (see *Volume 4, Chapter 12*). Invertebrate seafood, such as shrimp, lobster and other crustaceans and molluscs can contain quite high concentrations of carotenoids. In crustaceans this is often astaxanthin present as carotenoprotein complexes; the red carotenoid is released by cooking. The highest concentration of carotenoids is usually in the shell or integument which is commonly discarded before eating. Some products, including eggs (roe), can provide significant amounts in the diet, however. There is a great structural diversity of carotenoids in seafood; in most cases the possible biological activity of these has not been tested. The intake of these foods in a normal diet is generally small, and not of great significance. These carotenoids are generally not detected in human blood.



astaxanthin (**404-406**)



canthaxanthin (**380**)

4. Good sources

Many food composition tables are available in the literature [1,4,14,25-28] and references to carotenoid compositions of food in various parts of the world are given in reviews [24,29,30]. Extensive compilations of tabulated data can be found on the internet [31,32]. Evaluation of the data leads to the conclusions summarized in Table 1 about good sources of β-carotene and other carotenoids of nutritional interest.

Knowledge of carotenoid content is only part of the story, however. The efficiency with which the food is digested and the carotenoid released, solubilized, absorbed, transported and metabolized, *i.e*. 'bioavailability', is another key factor (see *Chapters 7* and *8*). The balance between content and bioavailability must always be considered.

Table 1. Good food sources of the nutritionally important carotenoids β-carotene, β-cryptoxanthin, lutein, lycopene and zeaxanthin, giving an indication of the likely carotenoid content. Precise values are not given but the content is indicated as a range, as follows. Low: 0 - 0.1 mg/100 g; Moderate: 0.1 - 0.5 mg/100 g; High: 0.5 - 2 mg/100 g; Very high: >2 mg/100 g.

Common name	**Latin name**	**Content**
β-Carotene		
Apricot	*Prunus armeniaca*	High - very high
Broccoli	*Brassica oleracea (Italiaca)*	Very high
Brussels sprouts	*Brassica oleracea (Gemmifera)*	High
'Buriti'	*Mauritia vinifera*	Very high
Butter		Low
Carrot	*Daucus carota*	Very high
'Gac' oil	*Momordica cochinchinnensis*	Very high
Grapefruit	*Citrus paradisi*	Low - moderate
Green leafy vegetables		Moderate - very high
Guava	*Psidium guajava*	Moderate
Kale	*Brassica oleracea (Acephala)*	Very high
'Karat' banana	*Musa troglodytarum*	High
Lettuce	*Lactuca sativa*	Moderate - high
Loquat	*Eriobotrya japonica*	Moderate
Mango	*Mangifera indica*	High - very high

β-Carotene (continued)		
Orange and juice	*Citrus* spp. and hybrids	Low - moderate
Papaya	*Carica papaya*	Moderate
Pea	*Pisum sativum*	Moderate
Peach	*Prunus persica*	High
Pepper (red, orange, green)	*Capsicum annuum*	High
Red palm oil	*Elaeis guineensis*	Very high
Spinach	*Spinacia oleracea*	Very high
Squash/pumpkin	*Cucurbita* spp.	Low - high
Sweet potato	*Ipomoea batatas*	Very high
Tangerine	*Citrus* spp. and hybrids	Low - moderate
Tomato	*Lycopersicon esculentum*	Moderate
Tomato, 'high-beta'		Very high
Tree tomato	*Cyphomandra betacea*	Moderate
West Indian cherry	*Malpighia glabra*	High

β-Cryptoxanthin		
Loquat	*Eriobotrya japonica*	Low - moderate
Papaya	*Carica papaya*	Moderate - high
Pepper (red, orange)	*Capsicum annuum*	Moderate
Persimmon	*Diospyros kaki*	High
Pitanga	*Eugenia uniflora*	High
Squash/pumpkin	*Cucurbita maxima*	Moderate - high
Tangerine	*Citrus* spp. and hybrids	Moderate - high
Tree tomato	*Cyphomandra betacea*	Moderate - high
West Indian cherry	*Malpighia glabra*	Low

Lutein		
Broccoli	*Brassica oleracea (Italica)*	Very high
Egg yolk		Moderate - high
Green leafy vegetables		Very high
Pepper (yellow, green)	*Capsicum annuum*	Very high
Squash/pumpkin	*Cucurbita* spp.	Moderate - very high

Lycopene		
Apricot	*Prunus armeniaca*	Low
Carrot (red)	*Daucus carota*	High
Grapefruit (red)	*Citrus paradisi*	Moderate - high
Guava	*Psidium guajava*	High
Papaya	*Carica papaya*	Moderate - high

Lycopene (continued)		
Persimmon	*Diospyros kaki*	Low - high
Tomato	*Lycopersicon esculentum*	Very high
Water melon	*Citrullus lanatus*	High - very high
Zeaxanthin		
'Buriti'	*Mauritia vinifera*	High
Chinese wolfberry 'Gou Qi Xi''	*Lycium chinensis*	Very high
Pepper (orange, red)	*Capsicum annuum*	Very high
Persimmon	*Diospyros kaki*	Moderate
Squash/pumpkin	*Cucurbita* spp.	Moderate
Sweetcorn	*Zea mays*	Moderate

5. Additives, colourants

β-Carotene and other synthetic or natural carotenoids or carotenoid-rich extracts are widely used as additives to colour processed food, drinks, confectionery, icecream *etc*. They are normally present in quite small amounts but in some cases the concentration can be significant. The concentration of β-carotene in some orange-flavoured drinks can be high enough to cause carotenodermia in people who drink large amounts.

C. Effects of Environmental Conditions and Cultivation Practice

Over more than 50 years there have been many studies of effects of conditions on carotenoid (often only β-carotene) content of green leaves, many with grasses and other forage plants. Analytical studies are reported from many parts of the world with many different species. The main findings have been summarized and discussed [4]. The results are very variable and sometimes conflicting. Because of variability of experimental design, analytical methods and species used, the precise numerical figures often reported and numerical comparisons between studies are of little real value. It is safe to say, however, that, as well as strongly affecting crop yield/productivity overall, *e.g.* the number and size of leaves, environmental conditions and cultivation practice also influence carotenoid content of leaves, since this is related to photosynthetic efficiency and density of chloroplasts.

In general, it can be concluded that optimal conditions that produce strong growth and healthy plants are consistent with good carotenoid content. This means soil that is of good quality and structure to allow strong root formation, and is well supplied with water, minerals and nutrients. Also, light is a significant factor. There may be differences in carotenoid composition and content between leaves or plants in sun and shade conditions, and excessive

light can cause a reduction in photosynthetic efficiency, *via* photoinhibition and photodamage. Light quality, *i.e*. intensity at different wavelengths, varies with altitude and can also be influential. For most plants there are optimal day and night temperatures. Heat stress, light stress and drought stress, and stress by pollution or salt are detrimental to carotenoid content, as they are to plant growth and health in general.

The age and maturity of plant tissues at harvest is a significant factor. The time of day at harvest can have a profound effect on water content, leading to apparent variations in carotenoid concentration based on fresh weight. Treatment post-harvest is also important, especially the conditions and time of transport, and storage in the market and in the home.

Information on particular crops can be obtained from extensive reviews, e.g. [4] and references therein. A good illustration is provided by the baby-food squash 'Northrup King' grown in the U.S.A. When this is grown in Michigan the concentration of carotenoids is much lower than in the same cultivar grown in North Carolina, presumably because of environmental factors, though the qualitative composition is similar [11].

D. Effects of Storage, Processing and Cooking

1. Stability and loss or retention of carotenoids

Carotenoids *in situ* in vegetables and fruit are usually more stable than when they are isolated, because of the protective effect of the special conditions within the tissues due to molecular interactions with proteins *etc*., molecular aggregation and crystallization, and the presence of natural antioxidants, including antioxidant enzymes, such as superoxide dismutase (SOD). Any disruption of the tissues, such as may occur during processing or cooking, or during natural aging, may lessen this protection, leaving the carotenoids exposed to damaging factors and susceptible to change. When fruits and vegetables are cut, chopped, shredded or pulped, this increases exposure to oxygen and may remove the physical barriers that normally keep apart the carotenoids and oxidizing enzymes such as lipoxygenase.

Knowledge of the properties of carotenoids suggests that when foods are being stored, processed or cooked the greatest losses and changes are caused by prolonged exposure to air, strong light, high temperature or acid. To minimize destructive effects, prolonged heating and exposure to strong light and air should be avoided.

Transportation, storage and processing of foods must be optimized to prevent or reduce loss of quality and to preserve nutritional benefits. Some losses are unavoidable, *e.g*. removal of undesirable though carotenoid-rich peel or skin. Carotenoids may also be lost or altered during processing and storage, by enzymic or non-enzymic oxidation and by geometrical isomerization, rearrangement or other reactions. These factors can be addressed and monitored during industrial processing, but not during home preparation, where losses can be considerable and more difficult to control.

The losses or changes may be balanced by the improved bioavailability when the food structure has been weakened (*Chapter 7*).

The stability of carotenoids varies between different foods, even when processed and stored in the same way, so conditions that maximize carotenoid retention have to be determined for each individual case.

Analytical results may seem to indicate that carotenoid concentration increases during cooking or thermal processing. These erroneous results are likely to be analytical artefacts resulting from, for example, unaccounted loss of water or leaching of soluble solids during processing, so that calculation of carotenoid content on a fresh weight or a dry weight basis is not comparable to that of the fresh raw material. In fruits and root crops, carotenoid biosynthesis may continue after harvest and the carotenoid content actually increase, as long as the tissues are not damaged. Green vegetables, however, commonly lose carotenoid on storage, especially under conditions, such as high temperature, which favour wilting.

2. Storage, cooking and processing

There have been many experimental studies of effects of storage, processing and cooking of many different foods. Details of particular examples are given in a number of specialized surveys [1,14,25,33], which include extensive lists of references to the original literature. The main general findings and conclusions are summarized below.

a) Transport and storage

Many carotenoid-containing foods are seasonal. To make the products available all the year round, they must be harvested at the peak time and then stored or processed under conditions that preserve them and their carotenoid content. Harvested crops are often transported to the market, sometimed over long distances.

Sun-drying is a cheap and easy traditional method of food preservation in poor regions but the exposure to air and sunlight is particularly destructive of carotenoids. Protection from direct sunlight helps to lessen the losses.

Freezing (the more rapid the better) and storage frozen generally preserve the carotenoids but subsequent slow thawing, especially of unblanched products, can be detrimental.

The short heat treatment of blanching may cause some losses but it inactivates oxidative enzymes and thus prevents further greater losses later.

Packaging with exclusion of oxygen (vacuum or inert atmosphere) and storing at low temperature and protected from light preserves the carotenoid content. The presence of natural antioxidants, the addition of antioxidants, or treatment with bisulphite as a preservative may also reduce the extent of degradation.

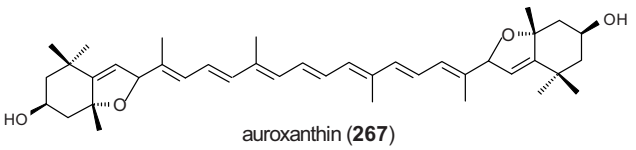
b) Cooking and processing

Not surprisingly, more drastic methods of cooking, *e.g*. for longer times or at higher temperatures, lead to greater losses. Prolonged boiling or deep-frying lead to the greatest changes. Baking and pickling are also detrimental.

With other methods, *e.g*. boiling for a short time (blanching), stir-frying, and microwave cooking, changes are small. Loss of carotenoids is usually small but heat provides energy for geometrical isomerization so the proportion of *Z* isomers increases. Carotenoid 5,6-epoxides are not stable and undergo rapid isomerization to the furanoid 5,8-epoxide form.

For any processing method, loss of carotenoid increases with longer processing time, higher temperature and cutting or pureeing. Exposure of green vegetables to severe heat treatment over extended periods, *i.e*. boiling for one hour, has been shown to result in complete destruction of carotenoid epoxides [2].

There are many publications in which losses of carotenoids are reported, usually for one or a small number of examples. Comparisons between studies are difficult and data may be somewhat conflicting. As examples, a study of the effects of cooking and processing on a number of yellow-orange vegetables, *e.g*. carrot, sweet potato and pumpkin, has demonstrated that the destruction of α-carotene and β-carotene as a result of heat treatment is about 8-10% [6]. Comparison of the extracts from several green vegetables (kale, Brussels sprouts, broccoli, cabbage, spinach), raw and cooked, [2] shows that some of their major chlorophyll and carotenoid constituents undergo some structural transformation. About 60% of the xanthophyll in Brussels sprouts is destroyed as a result of cooking [2]. In cooked kale the figure is about 68%. Lutein and zeaxanthin survive, though with some loss, whereas the epoxide violaxanthin is mostly destroyed or converted into the 5,8-epoxide auroxanthin (**267**); only 10% and 12% of the violaxanthin survives in cooked Brussels sprouts and kale, respectively. The same study reported no significant changes in the ratio of the *E* and *Z* isomers of neoxanthin, lutein epoxide and lutein.



auroxanthin (**267**)

The epoxycarotenoids are sensitive to heat, light, and trace amounts of acids, and readily undergo rearrangement, stereoisomerization, and degradation. The cooking process also degrades the chlorophylls. Losses of β-carotene are small (about 15%) and the heat treatment does not cause extensive stereoisomerization [2]. In several varieties of squash the esters have been shown to be more stable than the free carotenols, and the diesters more so than the monoesters [11]. Monoesters of violaxanthin were completely destroyed by cooking, but the diesters of violaxanthin survived to some extent. No significant change in the ratio of *E/Z*

isomers of violaxanthin diesters nor rearrangement of the esters to the 5,8-epoxide form as a result of cooking was indicated, in contrast to the unesterified violaxanthin. Lutein monoesters and diesters were also stable.

The quantitative loss and rearrangement of carotenoid 5,6-epoxides in cooked foods should not be of great concern since these compounds and their byproducts have not been detected in human serum or plasma [34,35].

As a result of various food preparation techniques, food carotenoids may undergo three main types of reactions, namely oxidation, rearrangement and dehydration. These reactions, which are described in the next Section, are dependent on the nature of the carotenoids, the food matrix, and the method of preparation.

3. Causes and mechanisms

The main changes that occur to carotenoid composition and content in foods during processing and cooking are oxidative breakdown and geometrical isomerization. Mechanisms of the isomerization and oxidative breakdown of carotenoids, in solution and in model systems, have been treated in *Volume 4, Chapters 3* and *7,* respectively. The same mechanisms apply to carotenoids in food, but the processes are modified by the special conditions *in situ*. There are many reports to show that carotenoids in foods vary in their susceptibility to degradation.

Oxidation, either enzymic or non-enzymic, is the main cause of destruction of carotenoids. Geometrical isomerization, which occurs particularly during heat treatment, increases the proportion of *Z* isomers and may alter the biological activity, but the total carotenoid content is not greatly changed. Conditions encountered during processing and storage may result in greater exposure to air, and so can induce greater losses than are caused by cooking. Also chopping or grinding can bring carotenoids into contact with degradative enzymes.

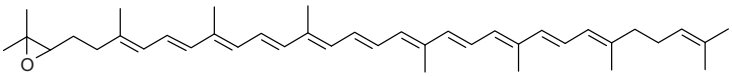
a) Oxidation

i) Enzymic. The main risk of enzyme-catalysed oxidative breakdown occurs during slicing, chopping or pulping of the fresh plant material, or when the food is allowed to wilt or become over-ripe, or in the early days of storage of minimally processed foods and unblanched frozen foods. The main breakdown is attributed to lipoxygenase enzymes. Oxidation of unsaturated fatty acids by these enzymes may be accompanied by bleaching (oxidative destruction) of carotenoids [36,37]. In the fresh healthy plant tissues lipoxygenase and carotenoids are in different locations. Only when the tissues are disrupted mechanically or as the tissues break down naturally can the enzyme come into contact with its carotenoid co-substrate. β-Carotene is usually most susceptible; with some leaves, up to 30% may be destroyed in seconds.

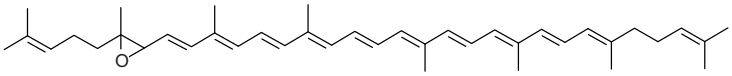
ii) Non-enzymic. Exposure to oxygen in the air during drying and processing leads to the generation of peroxides and oxidizing free radicals and can cause serious losses of

carotenoids. Conditions of sun-drying in air are particularly damaging. Even under less drastic conditions, however, once the generation of oxidizing species has been initiated, the process can continue to progress during storage, even at freezer temperatures, leading to increasing losses with time.

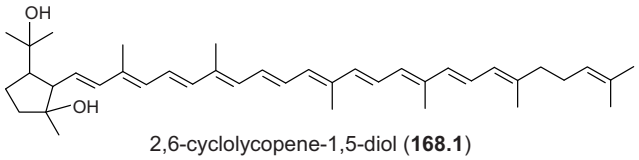
Oxidative degradation of carotenoids produces apocarotenals. The amount of these detected is small compared with the amount of carotenoid lost. The large amounts of apocarotenoids found in some *Citrus* fruit and juices are likely to be formed by specific, controlled enzymic reactions. During the storage and processing of tomatoes, oxidation of lycopene also produces small amounts of lycopene 1,2-epoxide (**217**) and 5,6-epoxide (**222**), the latter leading to the 2,6-cyclolycopenediols (**168.1**) that may be detected in serum [9,35].



lycopene 1,2-epoxide (**217**)



lycopene 5,6-epoxide (**222**)



2,6-cyclolycopene-1,5-diol (**168.1**)

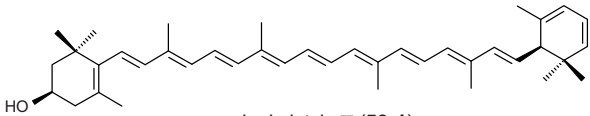
b) Geometrical isomerization

Geometrical isomerization is promoted by heat treatment and exposure to light, and may also result from exposure to acids. Increases of up to 40% in the proportion of *Z* isomers have been reported following heat treatment in the canning of several fruits and vegetables. The main *Z* isomer components found depend on the treatment and conditions. Isomerization of the Δ^{13} double bond has the lowest activation energy so the 13*Z* isomer is predicted to form most readily and to predominate if thermodynamic equilibrium has not been reached (*Volume 4, Chapter 3*). This has been shown to be the case in most heat-processed yellow, orange and red fruits and vegetables [38]. If thermodynamic equilibrium has been reached, it is predicted that the 9*Z* isomer should predominate. This has been reported in processed green vegetables, though chlorophyll-sensitized photoisomerization may be a factor in this. Lycopene in tomatoes is largely in a microcrystalline form and is comparatively resistant to isomerization [39]. In heat-processed tomatoes only about 5% of the lycopene is in the form of *Z* isomers, a

similar figure to that in the raw fruit. A major product of the geometrical isomerization of lycopene is the 5*Z* isomer, which was overlooked prior to HPLC studies [40].

c) Other changes

i) Rearrangement of 5,6-epoxides. Carotene and xanthophyll 5,6-epoxides are readily transformed into the corresponding 5,8-epoxides, *e.g*. violaxanthin (**259**) into auroxanthin (**267**). The change in absorption spectrum that accompanies this isomerization leads to loss of colour intensity. Carotenoid epoxides are not detected in the human body so these changes are unlikely to have any nutritional consequences.
ii) Dehydration. Carotenoids with an allylic hydroxy group are susceptible to dehydration under acid conditions. A good example of this is lutein (**133**). Its dehydration products, especially anhydrolutein II (2′,3′-didehydro-β,ε-caroten-3-ol, **59.1**), are detected in the HPLC profile of squash [12].



anhydrolutein II (**59.1**)

E. Conclusions and Recommendations

In both rich and poorer countries the aim is the same, namely to identify and increase the availability of the foods and products that give the highest amounts of the desired carotenoids in a form that is used efficiently, and to avoid losses of these carotenoids during storage, drying, processing and cooking.

1. Analytical data

a) HPLC

Modern HPLC analysis (*Chapter 2*) generates precise quantitative data about the sample being analysed. Based on this, extensive tables are available, listing concentrations of β-carotene and other carotenoids in a wide range of fruits, vegetables and other foods, including different varieties and strains. These tables provide a wealth of valuable information but a cautious and realistic approach is needed for the use and evaluation of the numerical data. The precise analytical figures refer to the particular sample that was actually analysed, and that

sample was grown, harvested and subsequently stored and processed in a particular way. This history is usually not reported. As explained earlier in this *Chapter*, so many factors can affect carotenoid content and composition, *e.g.* environmental conditions, cultivation practice, method and time of harvest, age and state of maturity of the sample at the time of harvest, length and conditions of storage. There is also great variation in the analytical methods and calculations used. Water content can vary widely and is usually not controlled, leading to considerable uncertainties when carotenoid content is referred to fresh weight. Even figures for different fruits taken from the same plant at the same time and extracted and analysed under identical conditions can show considerable variation. So, too much reliance should not be be placed on the precise figures listed. Presenting a range of values is more realistic. If a table gives a value of 148 μg/g fresh weight for β-carotene in a particular fruit or vegetable, an apparently similar sample of the same variety analysed under the same conditions must not be expected to give the same precise figure. Discrepancies for the same material grown and analysed in different parts of the world may be large. The figures are indicative, however, and in this way very useful. If a figure of 148 μg/g is listed in a table, it is realistic to expect that for any sample the content is likely to be in the range 100-200 μg/g. It is also safe to assume that a fruit reported to contain 148 μg/g should contain in the order of ten times as much carotenoid as a different example reported to contain 15 μg/g. The composition tables when used in a realistic way, therefore provide useful guidelines.

b) Visual assessment

The importance of visual assessment should not be overlooked. The human eye is a sensitive instrument and, when it is used in an informed way, direct observation can give a reliable assessment of colour (hue and intensity) allowing broad judgement of carotenoid composition and content from which possible good sources of carotenoids can be identified.

One can get much guidance simply by observation, before consulting tables or performing analysis. Thus, as a simple but valuable guideline, dark green leaves and vegetables have a higher chloroplast density and hence β-carotene and lutein content than paler green ones. Yellow-orange-red fruits and roots may be coloured by carotenoids, but alternatively the colour may be due to other pigments. This can usually be ascertained by checking the tables for that or a similar species. Carotenoids are not water-soluble; the other pigments, especially anthocyanins, are. This can be tested quickly and easily. As with green leaves, the strongest colour indicates the highest pigment concentration. Observation of the colour/hue is also useful. A yellow-orange colour indicates that a source may contain α-carotene and β-carotene and/or their hydroxy derivatives lutein and zeaxanthin. A red source may contain lycopene. This can be supported by the UV/Vis absorption spectrum of the total extract. Any source that looks promising is then analysed by HPLC (*Chapter 2*).

It is also easy to see if the colour varies between tissues, leaves or at different depths within the tissue, as a good indication of which samples should be analysed in detail.

c) Instrumental

Instrumental evaluation of colour intensity and hue as CIELAB coordinates by spectroradiometry or tristimulus colorimetry can be informative [41]. This determines three colour coordinates, namely L^* (luminosity or lightness), a^* (positive values indicate redness, negative values greenness) and b^* (positive values indicate yellowness, negative values blueness). For the yellow-orange carotenoids, a^* and b^* are both positive. From these measurements, two parameters can be calculated, namely c_{ab} (chroma) and h_{ab} (hue). This rapid method has been validated by correlation with HPLC results [42] and used, for example, to characterize various orange juices [43] and estimate their provitamin A value [44].

2. Some general conclusions

The number of carotenoids for which associations with health and biological activity have been studied is small. Generally, these are the only ones that are included in the food composition tables. The number of carotenoids eaten in a varied diet is much larger. The possibility that, in the future, other carotenoids may become of interest in relation to human health should not be overlooked; data may not be available on their occurrence and content.

In regions where vitamin A deficiency is still a real or potential problem, the requirement is specific: to obtain sufficient β-carotene and other provitamin A carotenoids from whatever sources are available and to minimize destructive effects during storage, processing and cooking. This is discussed in *Chapter 9*.

For tropical regions, local sources may not be covered in the tables. Unusual carotenoid-rich local sources strongly merit further study. Known examples are ‘buriti’ and ‘gac’ fruit, but exploration will surely reveal other interesting ones.

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